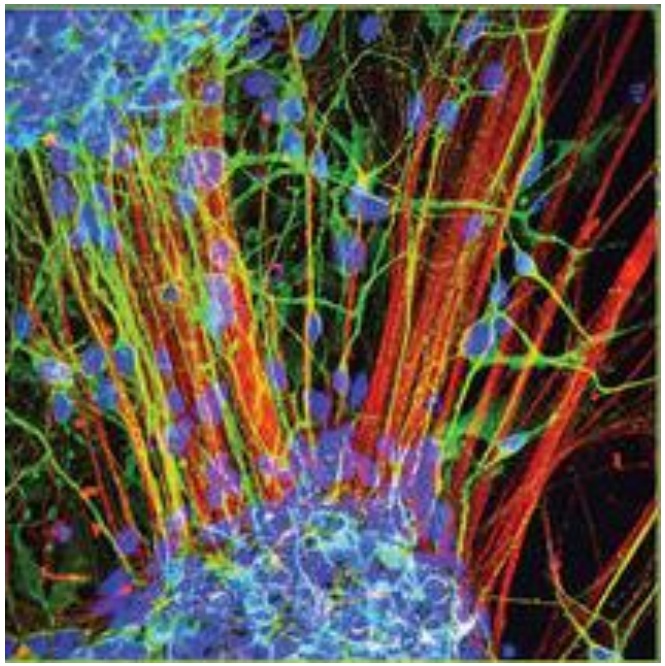
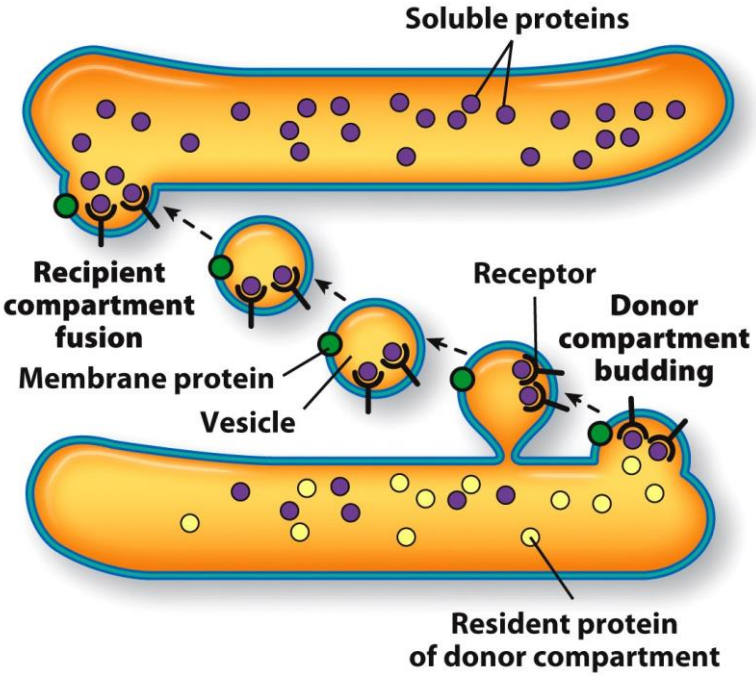


Biol 266 – Cell Biology

UNIT 1
"Cells and Organelles - II"

Membranes divide the cytoplasm of eukaryotic cells into distinct compartments.

The **endomembrane system** includes organelles such as the endoplasmic reticulum, Golgi complex, endosomes, lysosomes, and vacuoles functioning as part of a coordinated unit.



Inside vesicle, orientation remains the same

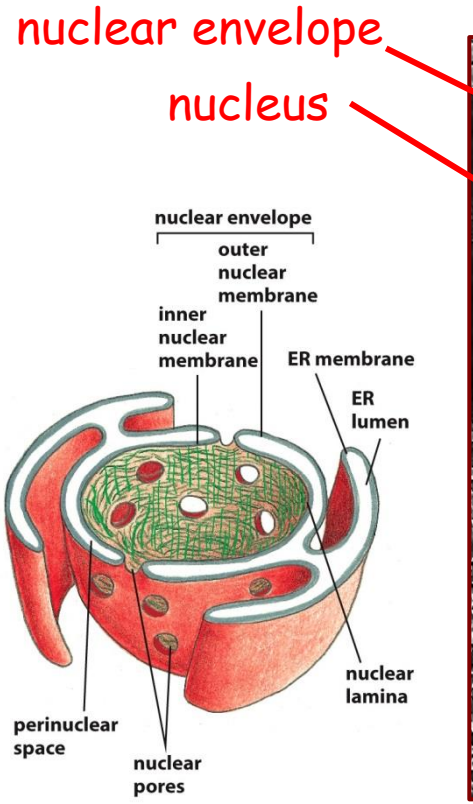
When material reaches the plasma membrane, lumen is secreted or exposed extracellularly

- Organelles of the endomembrane system are part of an integrated network in which materials are shuttled back and forth.
- Materials are shuttled between organelles in membrane-bound **transport vesicles**.
- Upon reaching their destination, the vesicles fuse with the membrane of the acceptor compartment.

The endoplasmic reticulum (ER) is a network of interconnected internal membranes that extends from the nuclear membrane throughout the cytoplasm

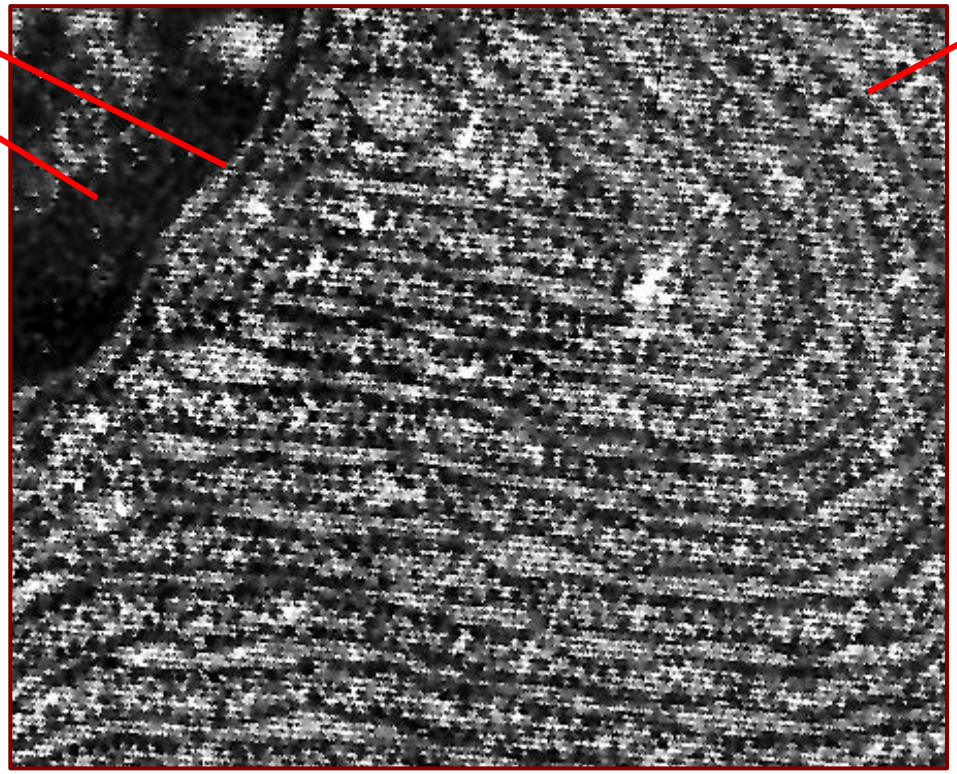
The ER membrane forms a continuous sheet enclosing a single internal space, the ER lumen

The ER is the largest organelle of most eukaryotic cells: the ER membrane is ~50% of all cell membranes and the ER lumen ~10% of the total cell volume

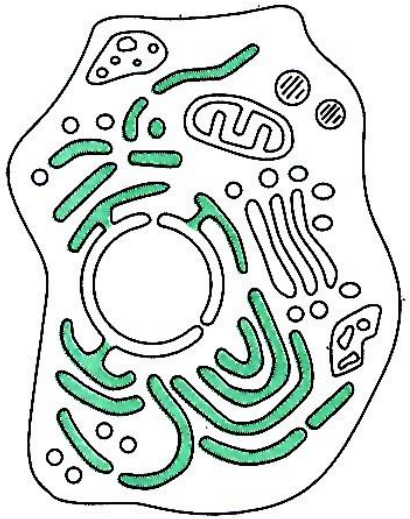


nuclear envelope

nucleus



ER



Three distinct types of ER perform different functions within the cell

1. The rough ER:

Ribosomes bound to the rough ER synthesize virtually all proteins to be secreted from the cell and proteins for most of the cell's organelles including:

a) the ER itself

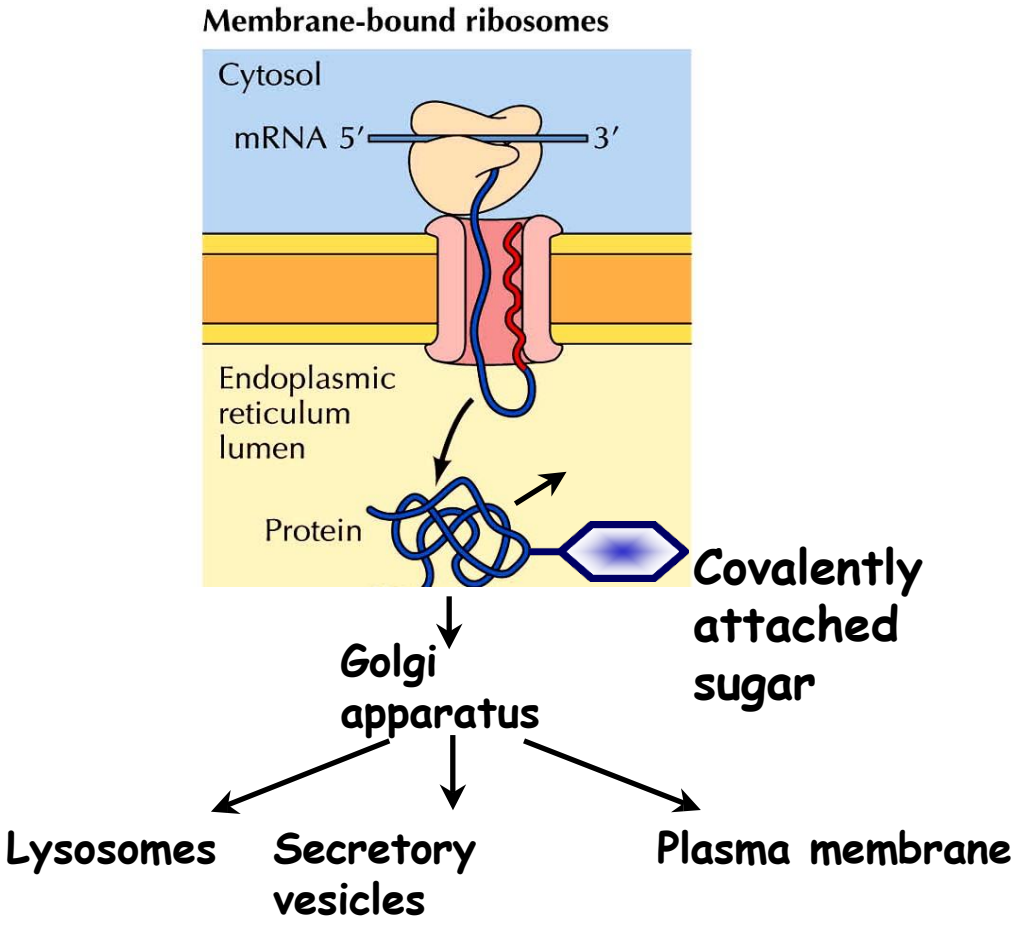
b) the Golgi apparatus

c) lysosomes

d) secretory vesicles

e) the plasma membrane

Many proteins are glycosylated within the ER by covalent attachment of sugars (glucose and mannose to their polypeptide chains)



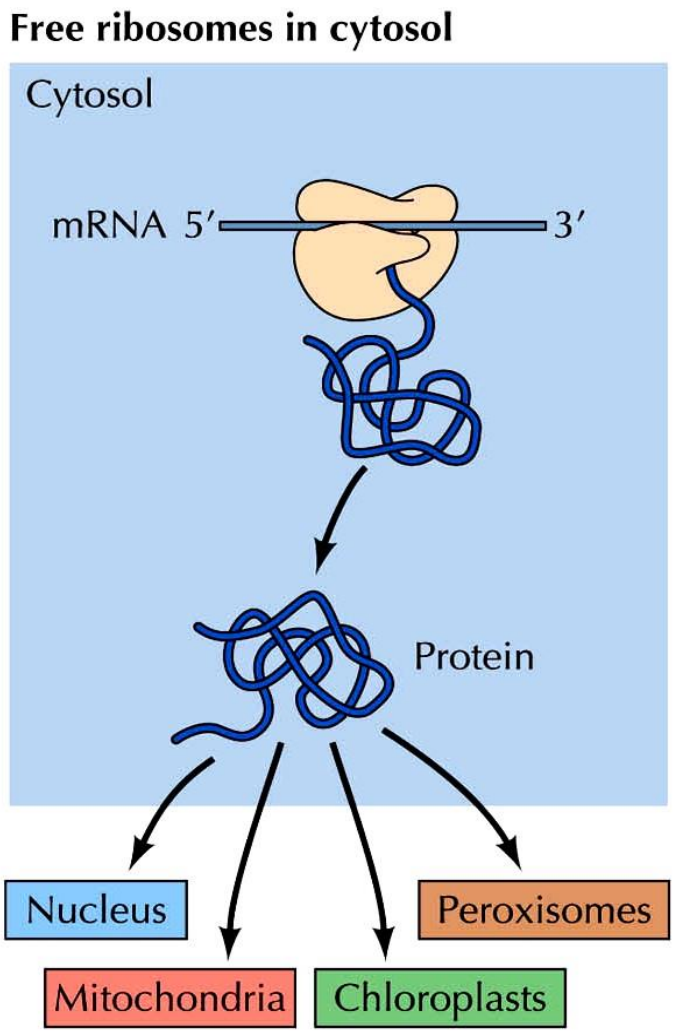
In contrast, proteins synthesized on free ribosomes in the cytosol either remain in the cytosol or are transported to:

a) the nucleus

b) mitochondria

c) chloroplasts

d) peroxisomes



2. The smooth ER

The synthesis of lipids, including fatty acids, cholesterol and phospholipids, occurs in the smooth ER

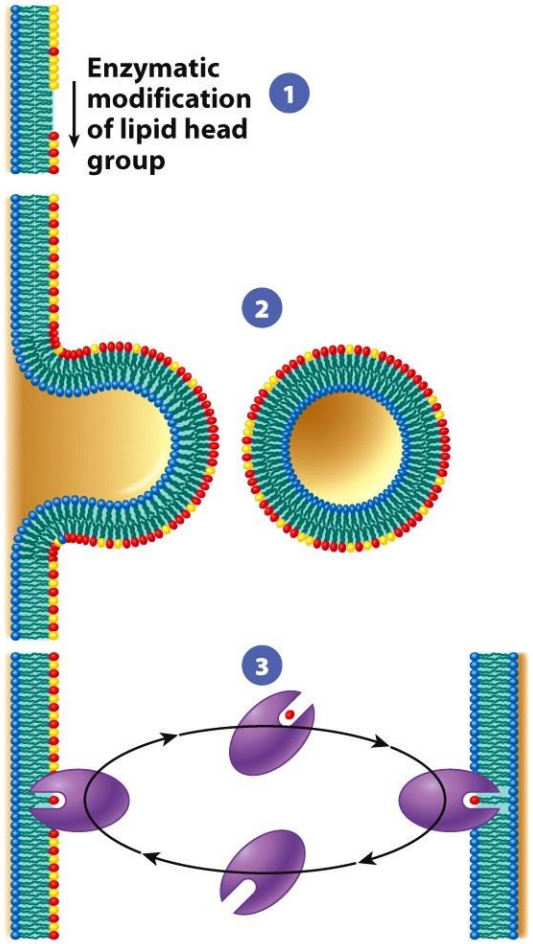
The smooth ER makes a major contribution to the lipid composition of membranes of all organelles by producing most of their lipids

After their synthesis in the smooth ER, lipids are transported from the ER to their ultimate destinations either in vesicles or by carrier proteins

SER has developed specialized functions in specific cells:

- In endocrine cells: synthesis of steroid hormones
- In liver cells: detoxification of various organic compounds (home of the P450 enzymes)
- In muscle cells: sequestration of calcium ion from cytoplasm of muscle cells

Modifying the lipid composition of membranes

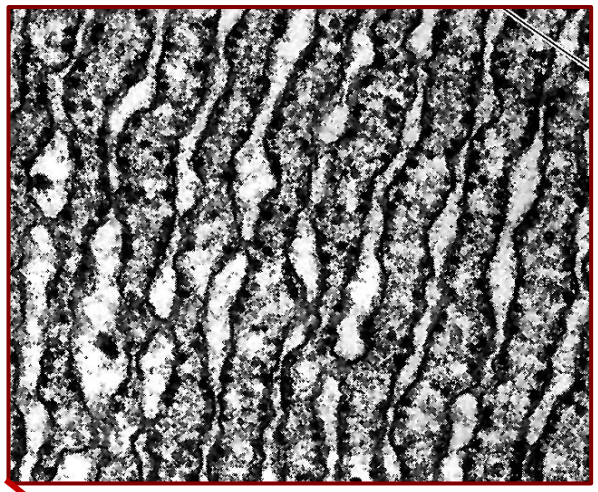


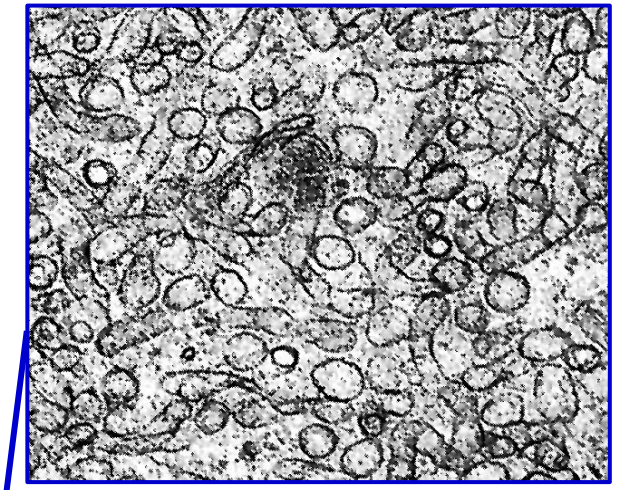
Schematic diagram showing three distinct mechanisms:

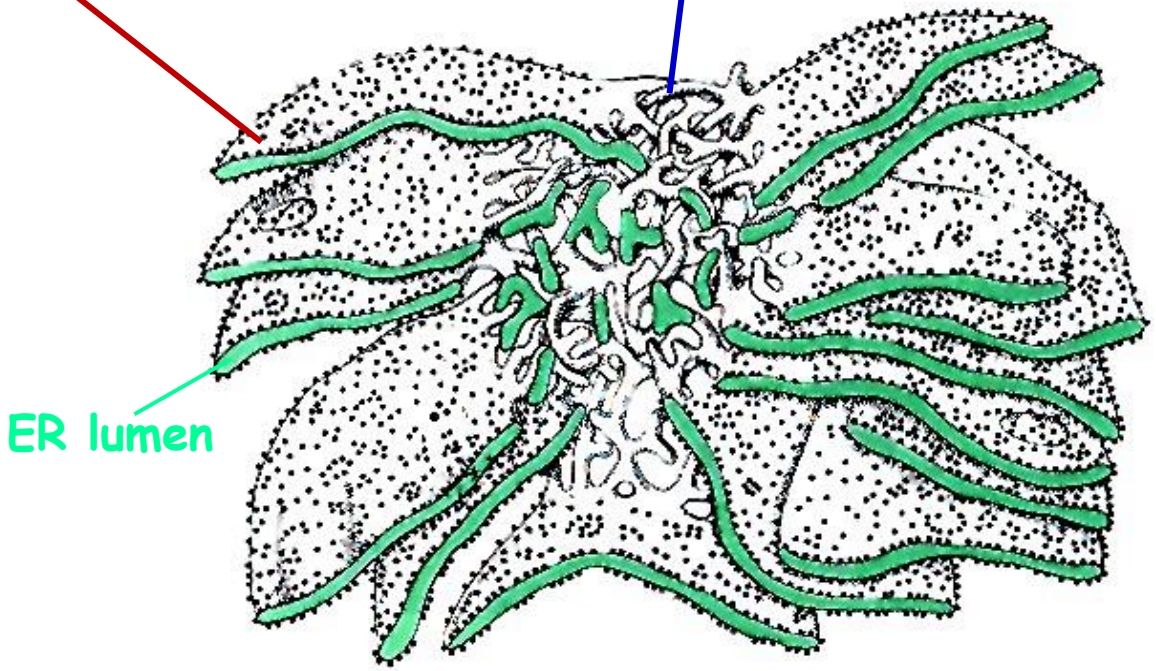
1. Enzymatic modification (head group)
2. Modification during vesicle formation
3. Modification by phospholipid transfer proteins

The rough ER forms oriented stacks of flattened cisternae

The smooth ER forms a fine network of tubules connected to the RER







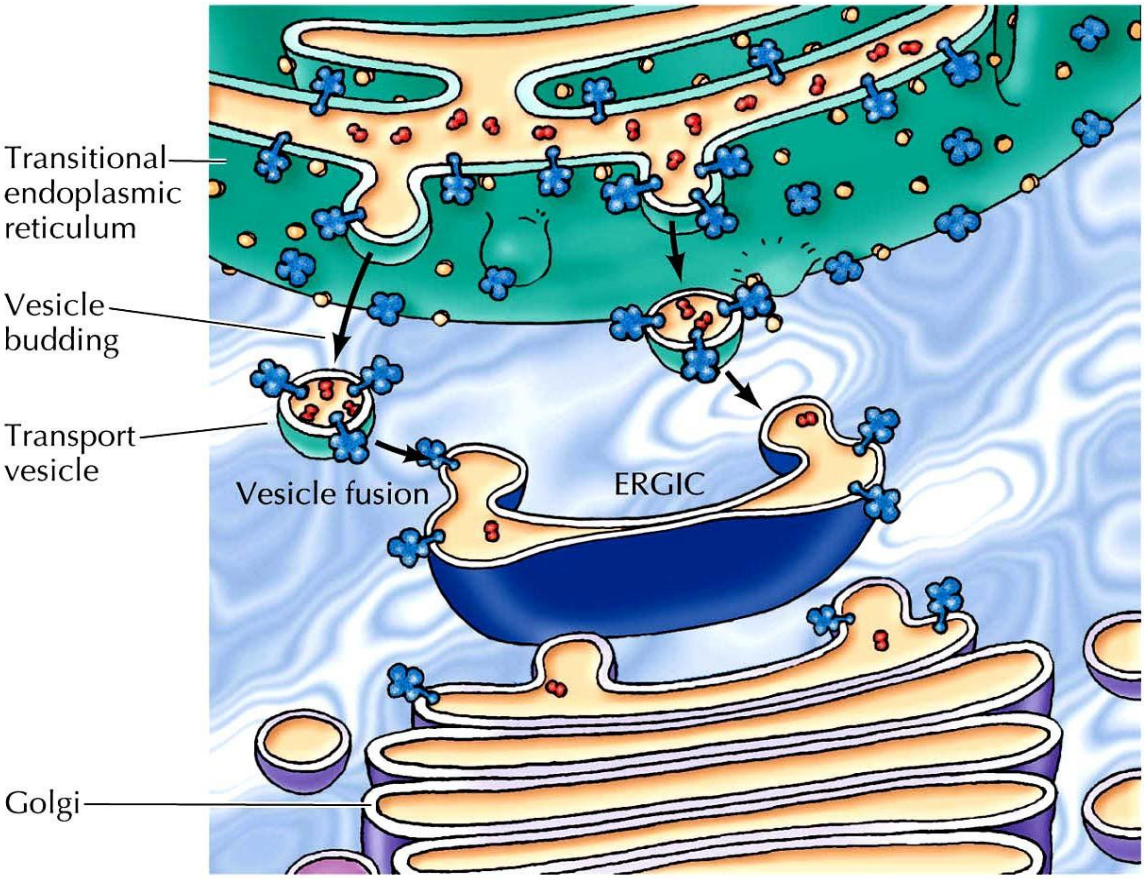
3. Transitional ER

A region of the ER where secretory vesicles exit the ER *en route* to the Golgi apparatus

Both proteins and lipids are exported from the ER in transport vesicles

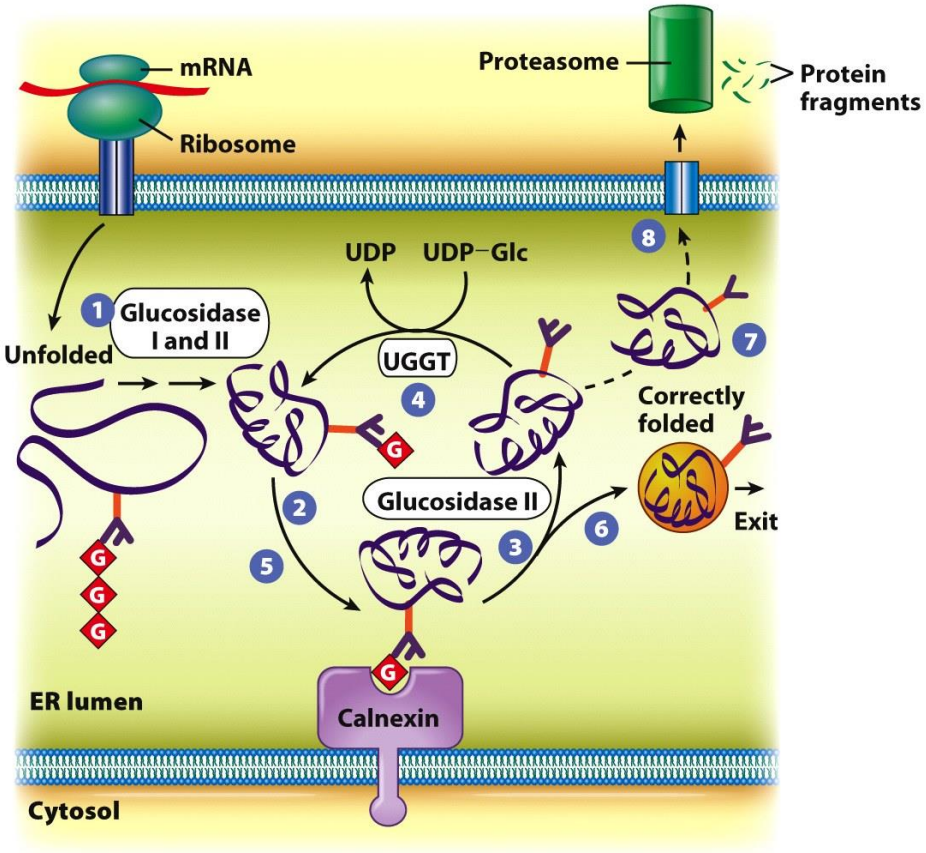
These vesicles bud from the transitional ER

After budding, these vesicles carry their cargo first to the ER-Golgi intermediate compartment (ERGIC) and then to the Golgi apparatus



One of the main functions of the ER once a protein has entered this organelle is called quality control, a system that monitors proper folding of a protein

1. After initial glycosylation in the ER, glucose residues are removed leaving a single terminal glucose
2. The protein then associates with chaperones including calnexin
3. The terminal glucose is removed and if the protein is not folded correctly….
4. ….the enzyme UGGT adds the glucose back….
5. ….and refolding is attempted again.
6. If the protein then folds it can be transported to the next compartment.
7. If it remains unfolded after several attempts, more sugars (mannose) are removed….
8. ….and the protein is dislocated from the ER to the cytoplasm to be degraded by the proteasome (ER-associated degradation (ERAD)).

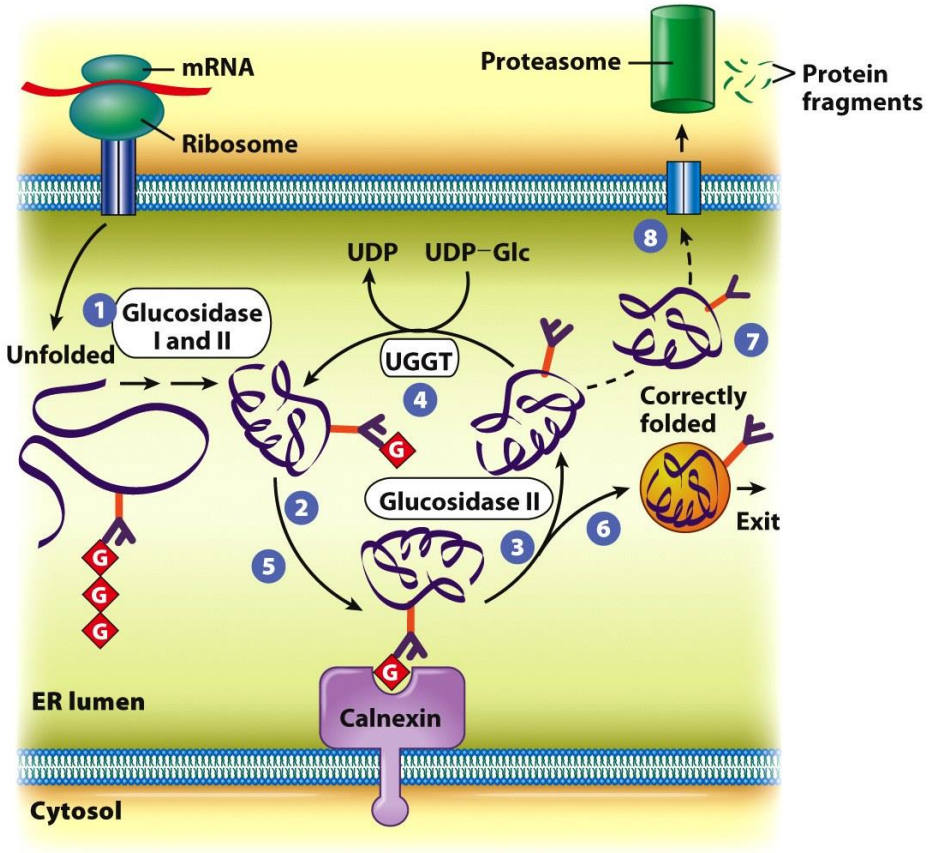


One of the main functions of the ER once a protein has entered this organelle is called quality control, a system that monitors proper folding of a protein

If too many unfolded proteins accumulate in the ER, then the unfolded protein response (UPR) is activated:

- Stops translation
- Degrades misfolded proteins
- Produces more chaperones

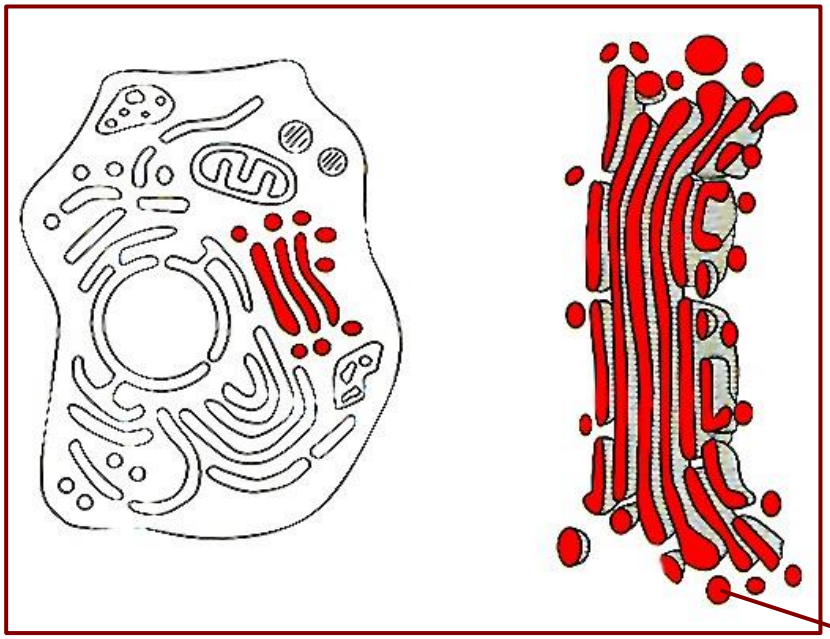
Prolonged UPR leads to apoptosis

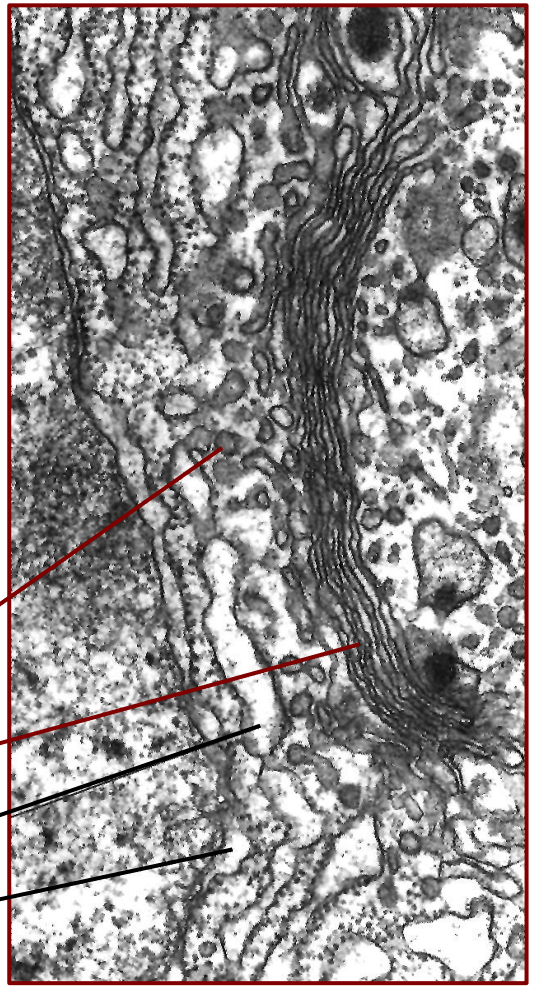


The Golgi apparatus (Golgi complex)

Morphologically the Golgi is composed of flattened membrane-enclosed sacs (cisternae) and associated vesicles

The Golgi apparatus is usually located near the cell nucleus

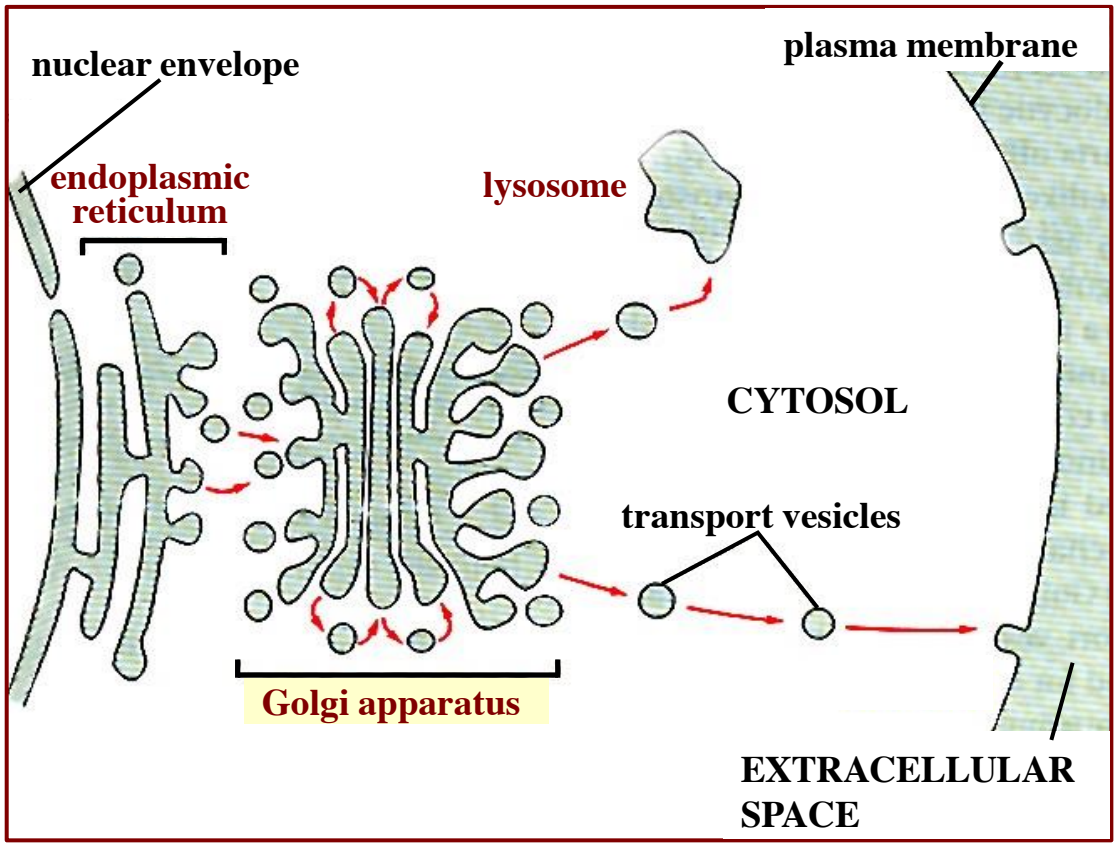




Functions of the Golgi

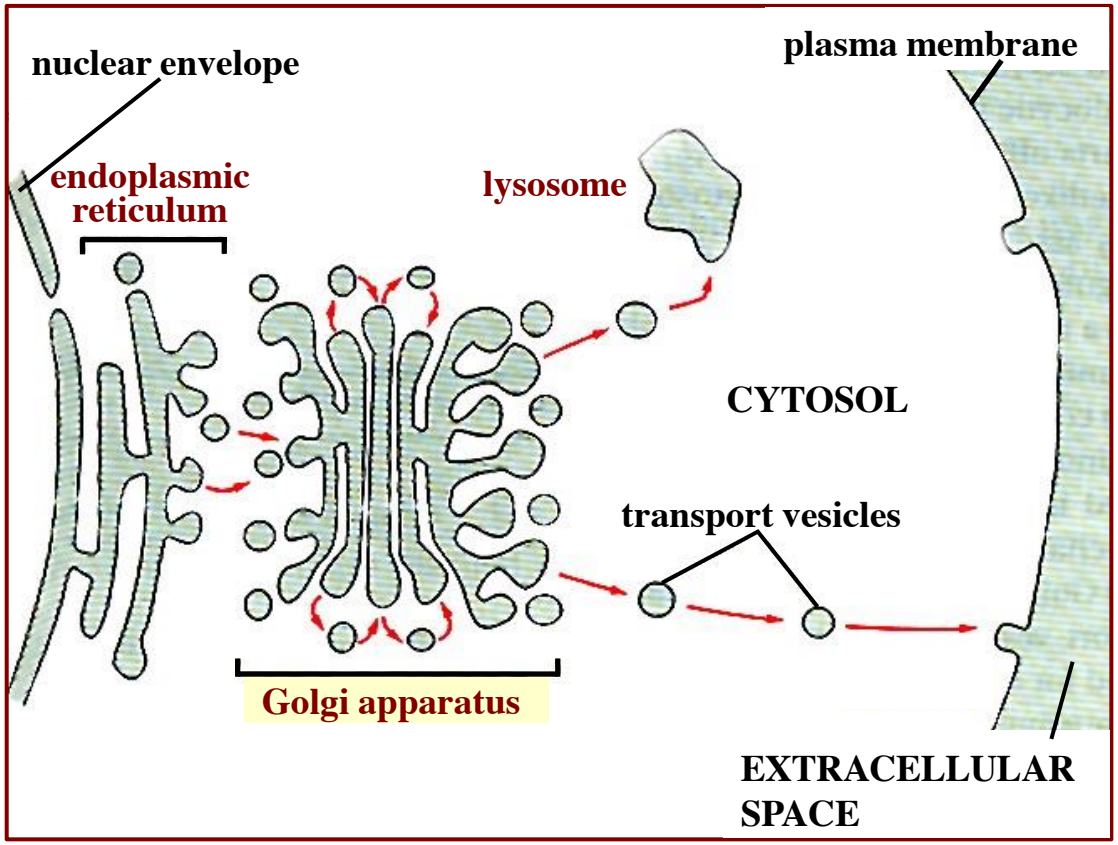
1. A factory in which proteins received from the ER:
   a. are further glycosylated

   b. are sorted for transport to their eventual destinations:
   - lysosomes
   - the plasma membrane
   - extracellular medium



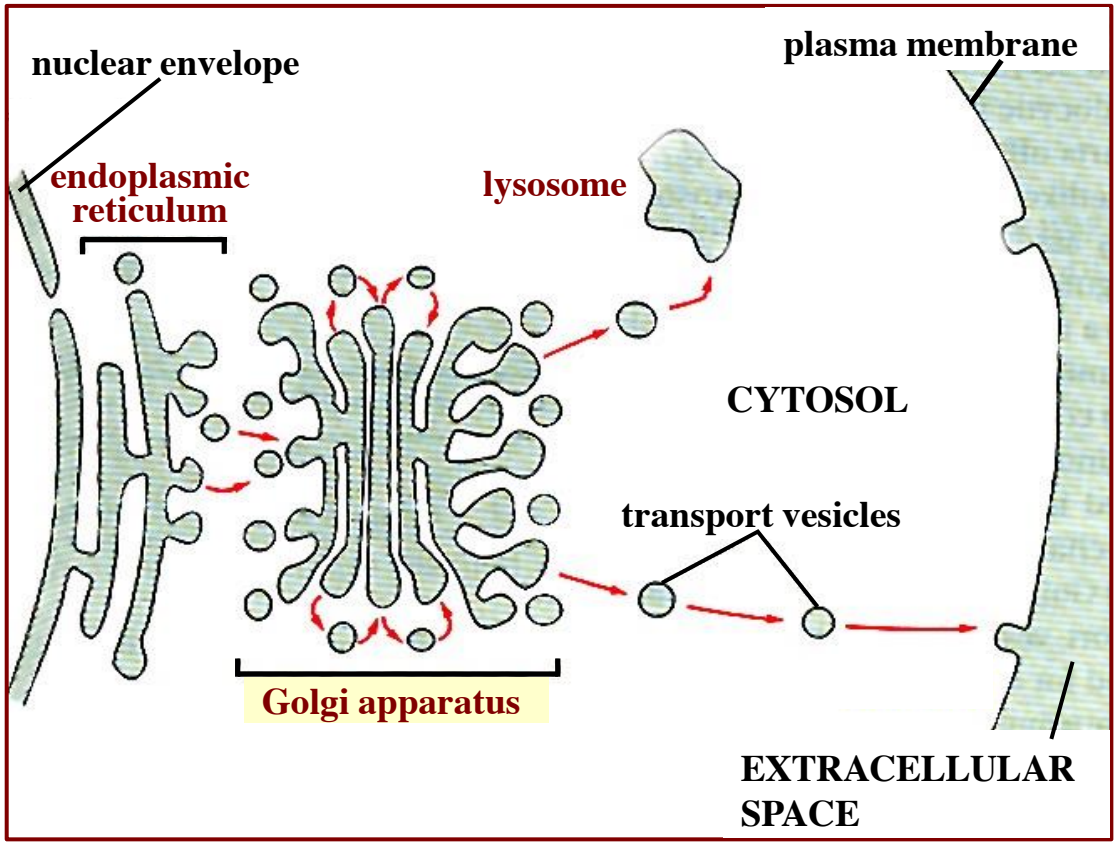
Functions of the Golgi

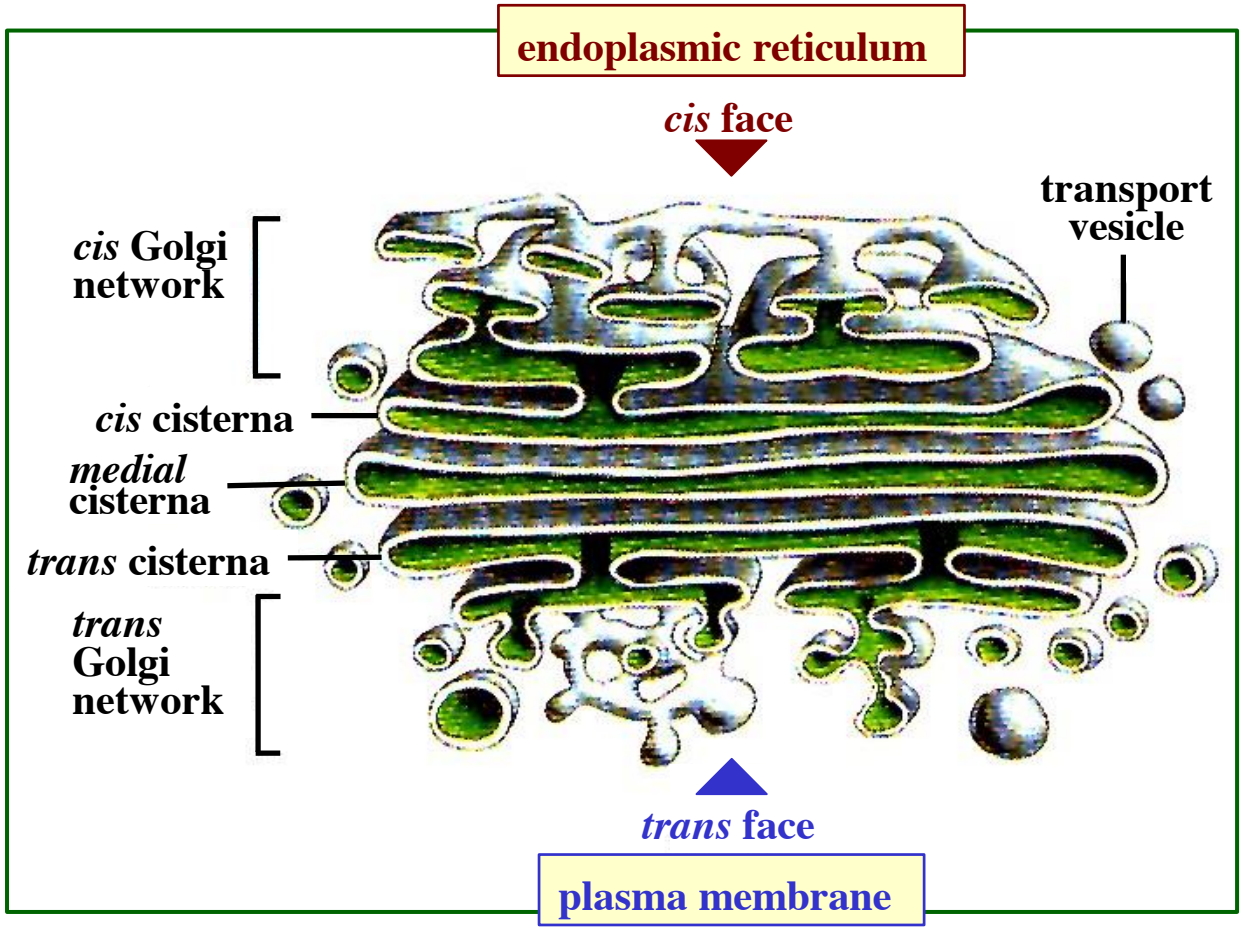
2. Some lipids, including glycolipids and sphingomyelin, are synthesized within the Golgi complex



Functions of the Golgi

3. In plant cells, the Golgi serves as the site at which the complex polysaccharides of the cell wall, including hemicelluloses and pectins, are synthesized



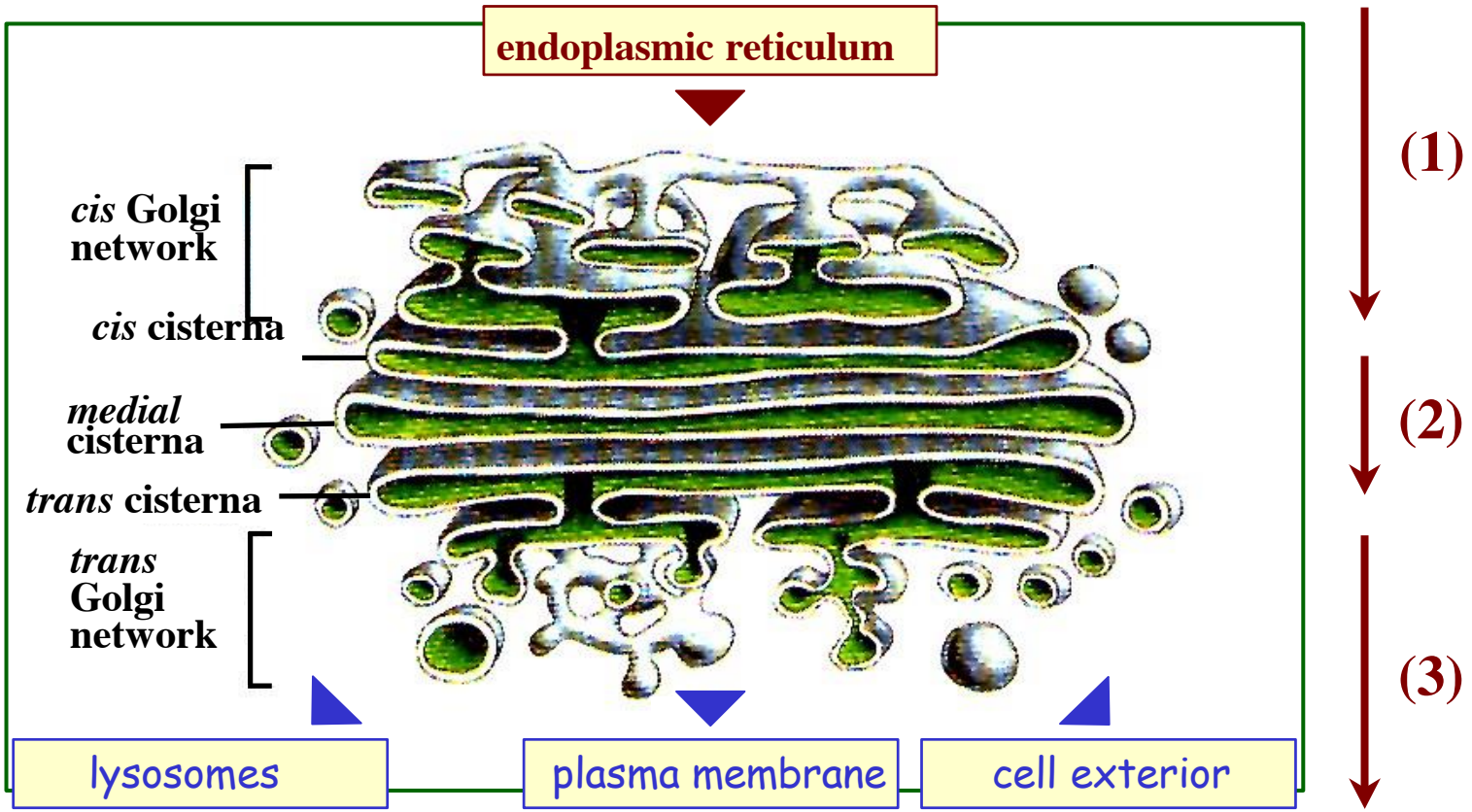


The Golgi consists of an ordered series of compartments

Each Golgi stack has two distinct faces:
(1) en entry, or ***cis***, face
(2)an exit, or ***trans***, face

The *cis* face is adjacent to the ER, while the *trans* face points toward the plasma membrane

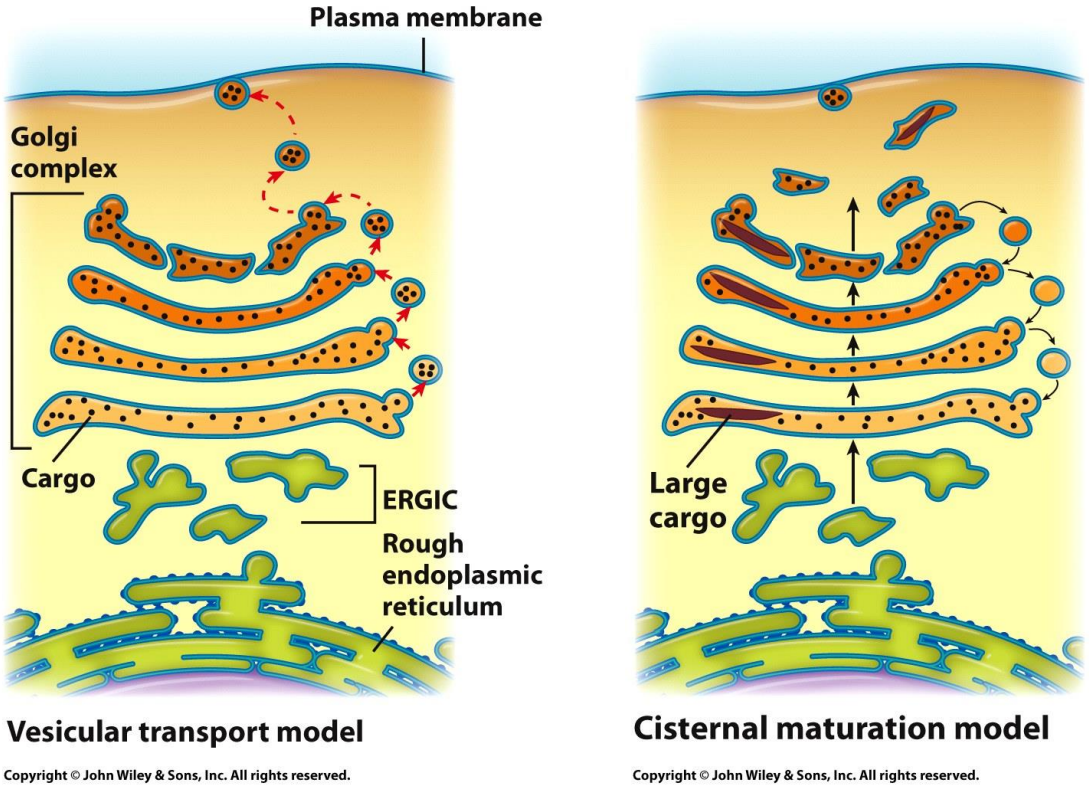
Five functionally distinct compartments:
1. the *cis* Golgi network
2. the *cis* compartment of the Golgi stack
3. the *medial* compartment of the Golgi stack
4. the *trans* compartment of the Golgi stack
5. the *trans* Golgi network



The movement of proteins within the Golgi

1. Proteins and lipids enter the *cis* Golgi network in transport vesicles from the ER
2. The proteins and lipids then progress to the *cis*, *medial* and *trans* compartments (cisterna) of the Golgi stack
3. The proteins and lipids then move to the *trans* Golgi network, which acts as a sorting and distribution center, directing molecular traffic of transport vesicles to lysosomes, the plasma membrane and cell wall exterior

The dynamics of transport through the Golgi complex



The movement of proteins within the Golgi

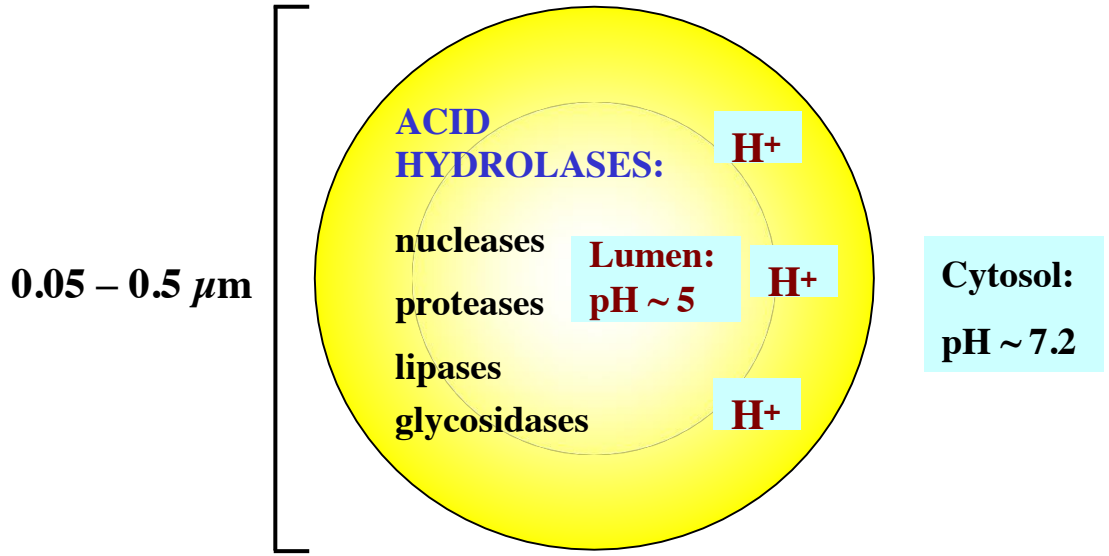
Vesicular transport: cargo is shuttled from the CGN to the TGN in vesicles

Cisternal maturation: each cisterna matures as it moves from the cis face to the trans face, mediated by vesicles traveling from trans face to cis face

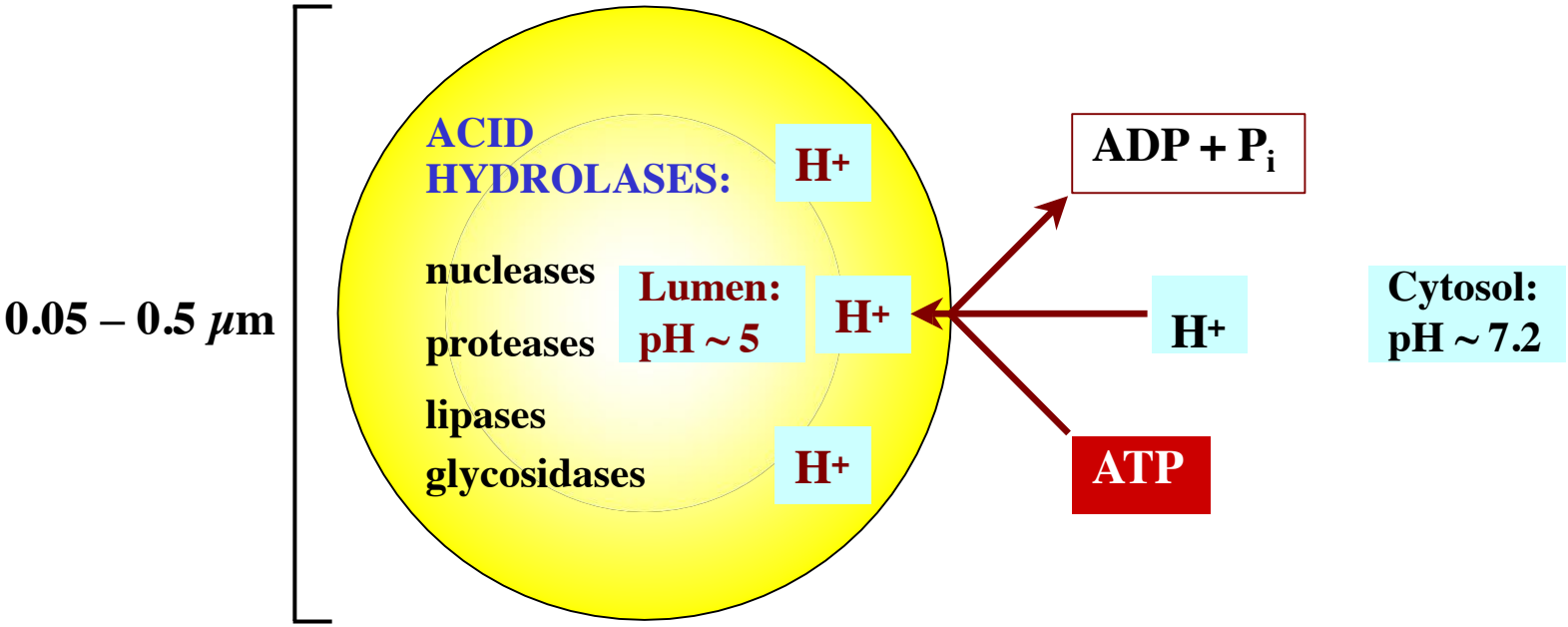
Lysosomes are membrane-enclosed organelles that contain about 50 different degradative enzymes that can hydrolyze:

1. nucleic acids (nucleases)
2. proteins (proteases)
3. lipids (lipases)
4. carbohydrates (glycosidases)

All lysosomal enzymes are acid hydrolases, which are active at acidic pH (~5) that is maintained within lysosomes but not at neutral pH (~7.2) characteristic of the cytosol



The lumen of the lysosome is maintained at acidic pH by a **H⁺ (proton) ATPase** in the membrane that pumps H^+ ions (protons) into the lumen



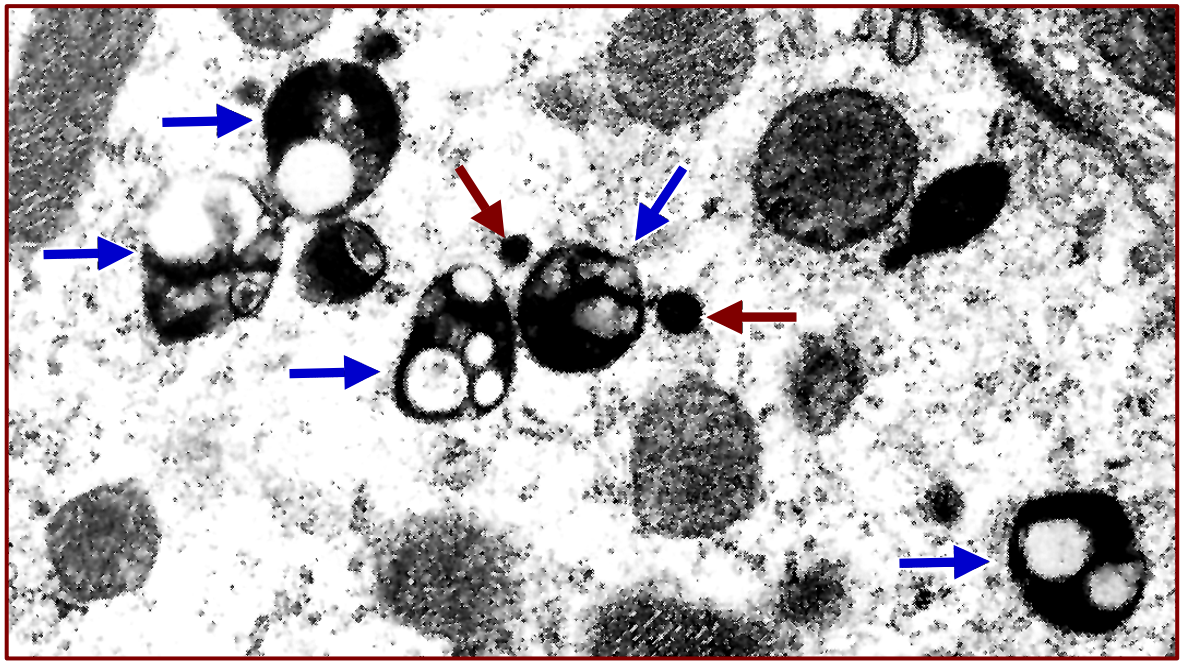
Integral membrane proteins of the lysosome are highly glycosylated. This is thought to shield the membrane from the degradative enzymes of the lysosome.

Lysosomes represent morphologically diverse organelles defined by the common function of degrading intracellular material.

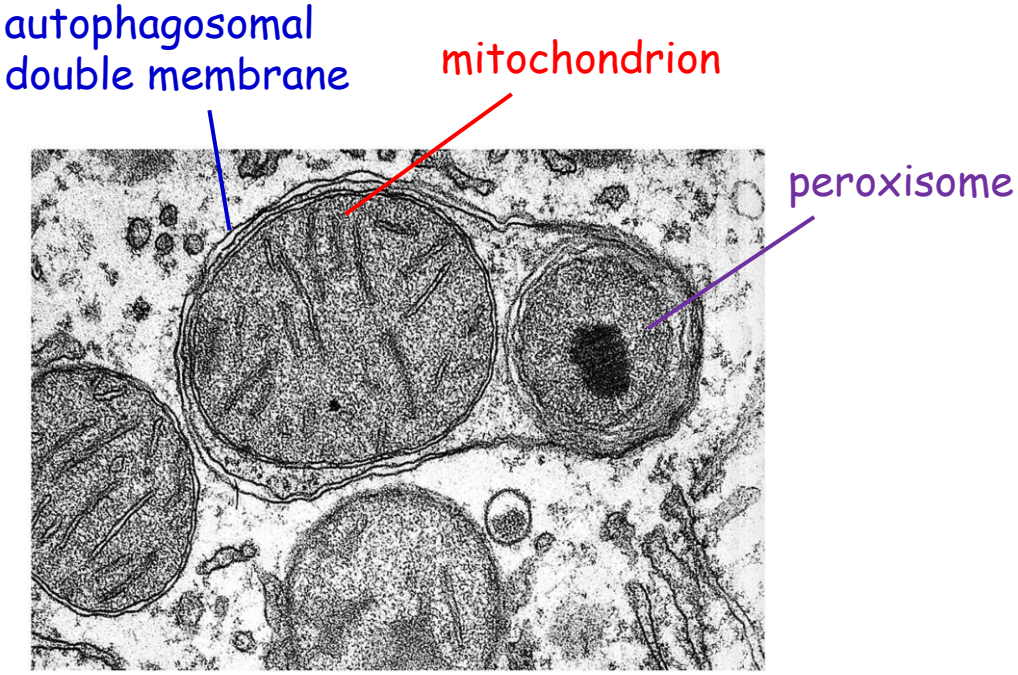
Lysosomes can display considerable variation in size and shape as a result of differences in the materials that have been taken up for degradation.

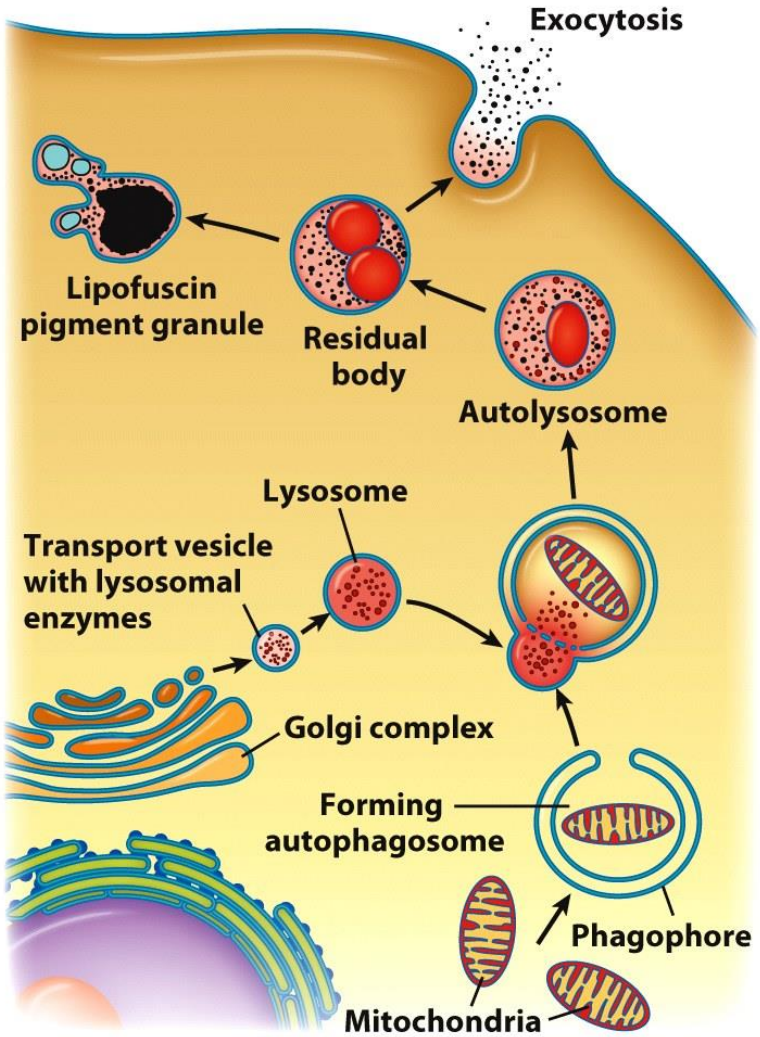
Primary lysosomes are roughly spherical and do not contain obvious particulate or membrane debris.

Secondary lysosomes, which are larger and irregularly shaped, result from the fusion of primary lysosomes with membrane-engulfed aged and defective organelles; they contain particles of membranes in the process of being digested (autophagy).



Example of autophagy of a mitochondrion (mitophagy)





Autophagosomes can contain:

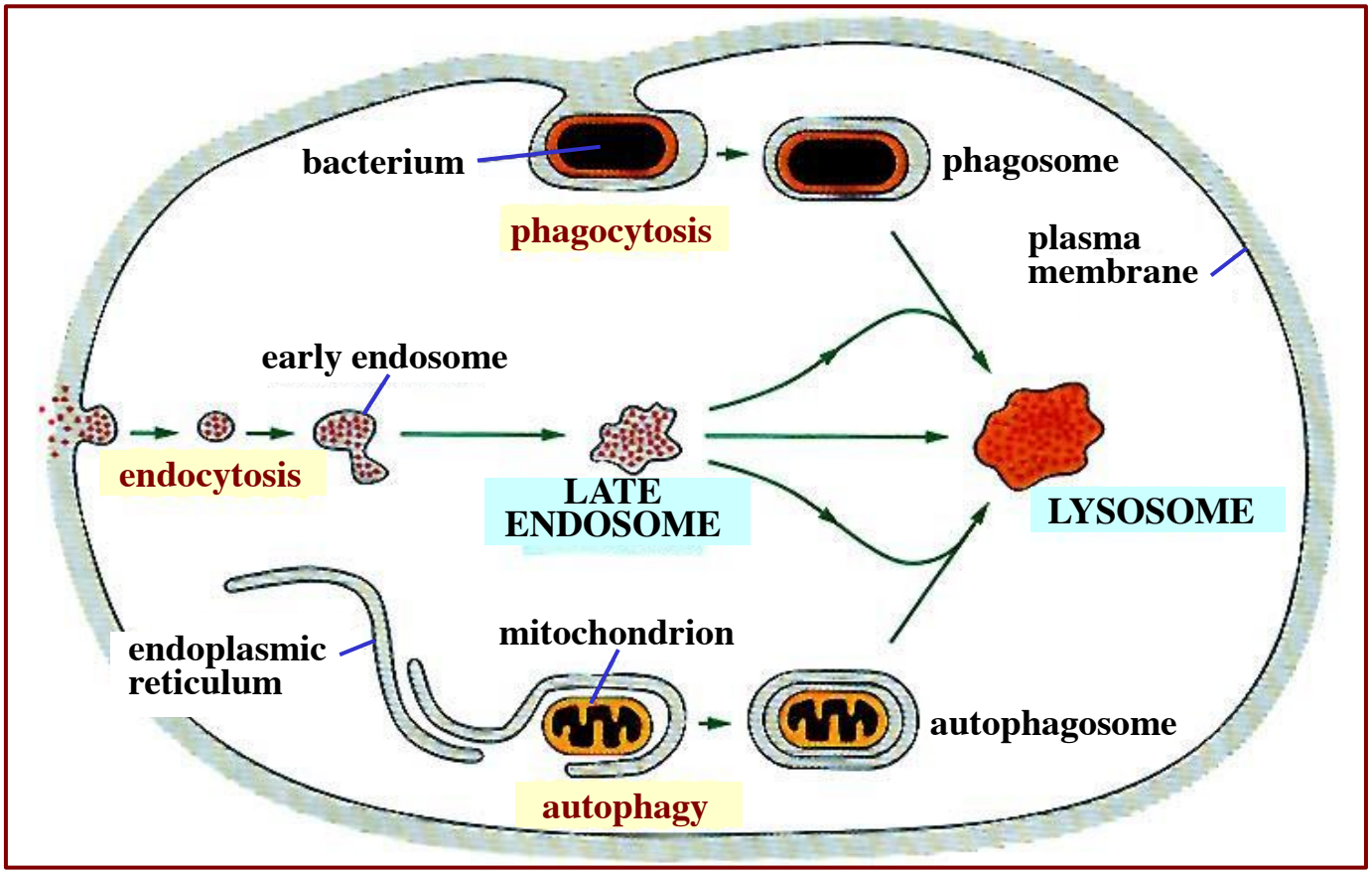
- Organelles
- Cytosolic proteins
- Lipids

Three pathways for delivering materials to lysosomes

The digestion of molecules taken up from outside the cell by endocytosis

The digestion of large particles, including bacteria, cell debris, and aged cells (taken up from outside the cell), by phagocytosis

The digestion of aged or defective organelles (the cell's own components) by autophagy



Lysosomal storage diseases

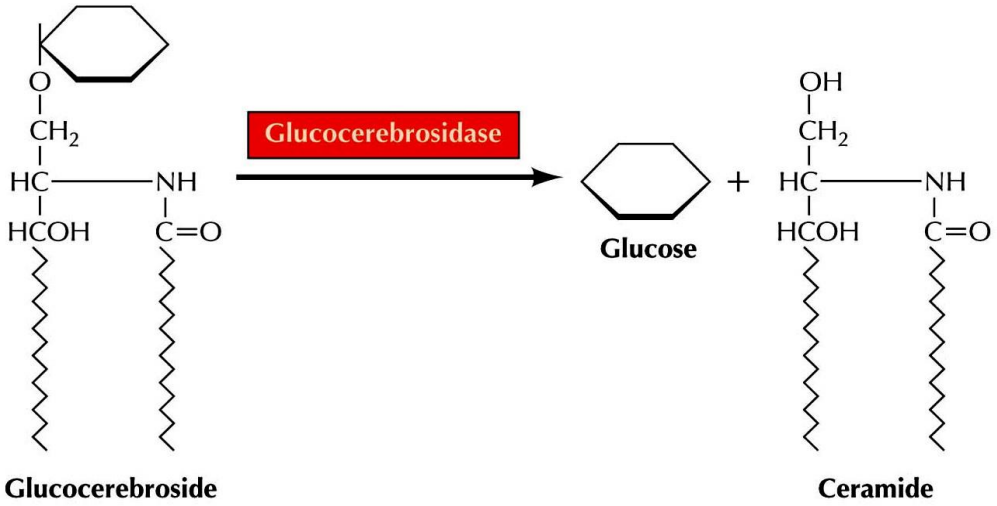
Mutations in the genes that encode lysosomal acid hydrolases are responsible for more than 30 different human genetic diseases

These diseases are called lysosomal storage diseases because undegraded material accumulates within the lysosomes of affected individuals

Table 1 Sphingolipid Storage Diseases

Disease	Enzyme deficiency	Principal storage substance	Consequences
G_{M1} Gangliosidosis	G_{M1} β-Galactosidase	Ganglioside G_{M1}	Mental retardation, liver enlargement, skeletal involvement, death by age 2
Tay-Sachs disease	Hexosaminidase A	Ganglioside G_{M2}	Mental retardation, blindness, death by age 3
Fabry's disease	α-Galactosidase A	Trihexosylceramide	Skin rash, kidney failure, pain in lower extremities
Sandhoff's disease	Hexosaminidases A and B	Ganglioside G_{M2} and globoside	Similar to Tay-Sachs disease but more rapidly progressing
Gaucher's disease	Glucocerebrosidase	Glucocerebroside	Liver and spleen enlargement, erosion of long bones, mental retardation in infantile form only
Niemann-Pick disease	Sphingomyelinase	Sphingomyelin	Liver and spleen enlargement, mental retardation
Farber's lipogranulomatosis	Ceramidase	Ceramide	Painful and progressively deformed joints, skin nodules, death within a few years
Krabbe's disease	Galactocerebrosidase	Galactocerebroside	Loss of myelin, mental retardation, death by age 2
Sulfatide lipidosis	Arylsulfatase A	Sulfatide	Mental retardation, death in first decade

Gaucher's disease is a lysosomal storage disease which results from a mutation in the gene that encodes a lysosomal enzyme required for the hydrolysis of the glycolipid glucocerebroside to glucose and ceramide.



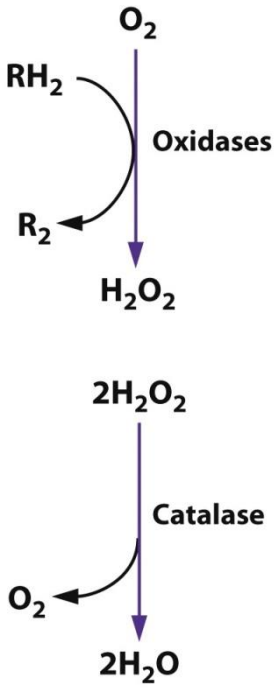
Treated using Cerezyme, a modified form of glucocerebrosidase that is taken up by endocytosis and delivered to the lysosome.

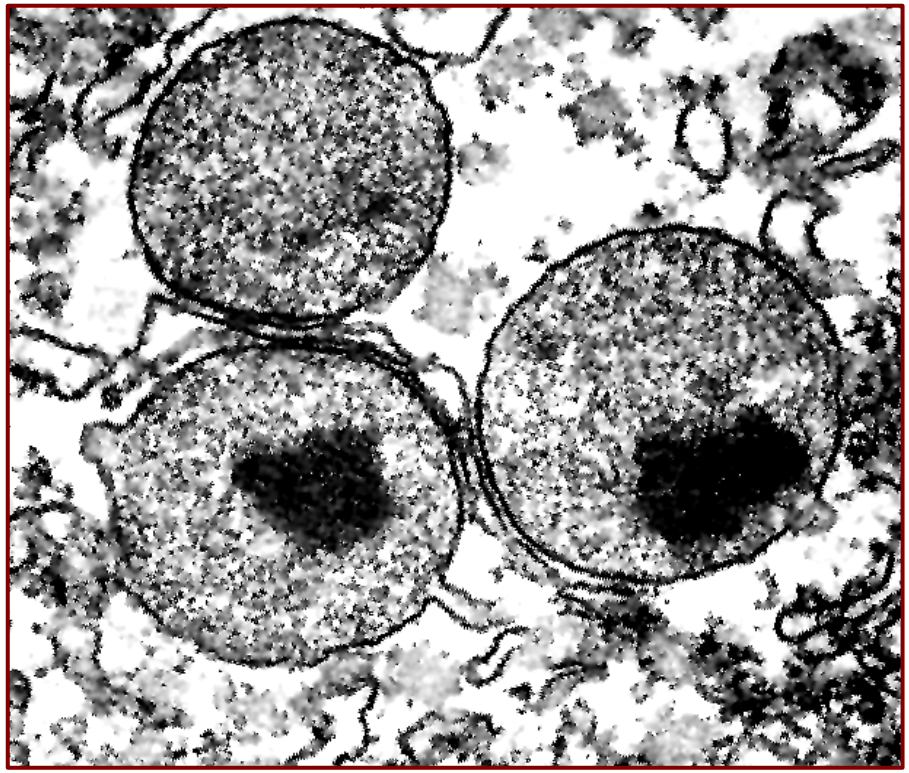
Other lysosomal storage diseases are treated similarly since endosomes ultimately fuse with lysosomes.

Some lysosomal storage disorders have been treated by inhibiting production of the accumulating substance.

Peroxisomes

Peroxisomes are the site of synthesis and degradation of hydrogen peroxide (H_2O_2), which is highly reactive and toxic. Hydrogen peroxide is produced during the oxidation of several substrates.



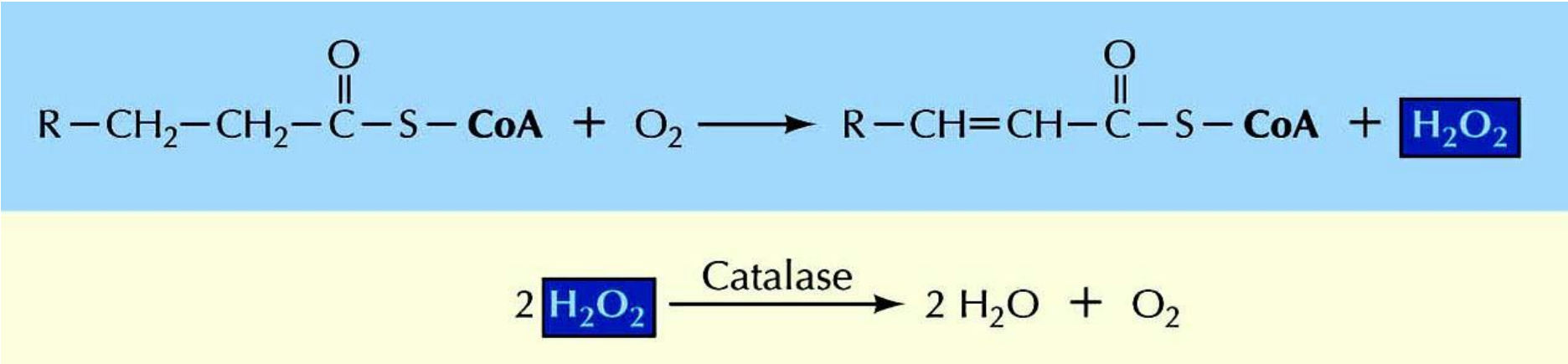


200 nm

Electron micrograph of three peroxisomes in a rat liver cell

Functions of peroxisomes

1. Oxidation of very long chain fatty acids:
   - leads to the production of harmful hydrogen peroxide
   - in contrast to mitochondrial fatty acid oxidation, peroxisomal oxidation of fatty acids is not linked to ATP formation
2. Decomposition of hydrogen peroxide



Hydrogen peroxide is used as a bleaching agent (hair, teeth). Reduced levels of catalase (during aging) may lead to graying of hair.

Functions of peroxisomes

3. Biosynthesis of plasmalogens, a lipid that is abundant in myelin (coating around nerve cell axons)

4. Conversion of stored fatty acids to carbohydrates in germinating seeds of plants, which is critical to providing energy for growth of the germinating plant (in this case the organelle is called the glyoxosome)

Similarities and differences between peroxisomes and mitochondria

- **Both are formed from a pre-existing organelle**
- **Both import preformed proteins from the cytosol**
- **Both oxidize fatty acids**

Mitochondria may even form vesicles that carry material to peroxisomes

- **Peroxisomes have a single phospholipid bilayer but mitochondria have two**
- **Peroxisomes do not contain DNA or ribosomes but mitochondria do**

Peroxisomal disorders

Zellweger syndrome: patients synthesize peroxisomal enzymes but cannot import them into the organelle. Thus, the peroxisomes appear as "ghosts". Neuronal phenotype.

X-linked adrenoleukodystrophy (X-ALD): peroxisomes do not import very long chain fatty acids (VLCFA) which then accumulate in the brain and impair the myelin. Has been treated successfully by gene therapy (adding back a functional VLCFA transporter).

The cytoskeleton

A network of protein filaments extending throughout the cytoplasm.

Functions:

1. Provides the structural framework of the cell:

   - determines cell shape
   - determines the general organization of the cytoplasm

2. Responsible for the movements of:

   - entire cells
   - organelles
   - transport vesicles
   - chromosomes during cell division

Three main kinds of cytoskeletal filaments

Microtubules

- 25 nm in diameter
- built of polymers of the protein tubulin
- intracellular transport, cell division, cell organization

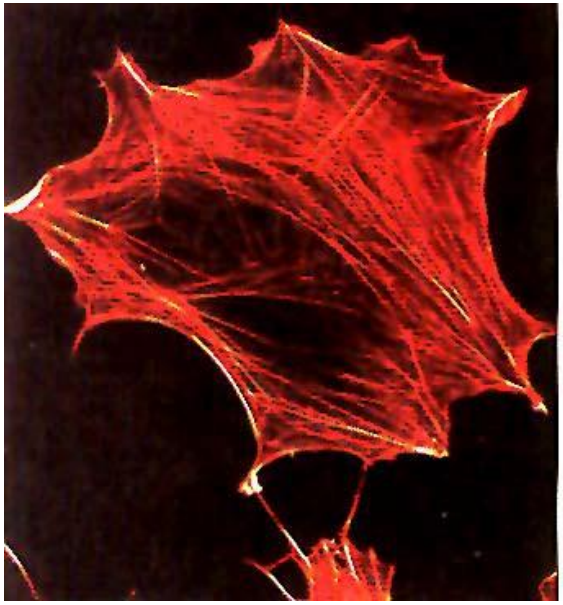
Actin filaments

- 8 nm in diameter
- built of the protein actin
- motility, contractility (in muscle)

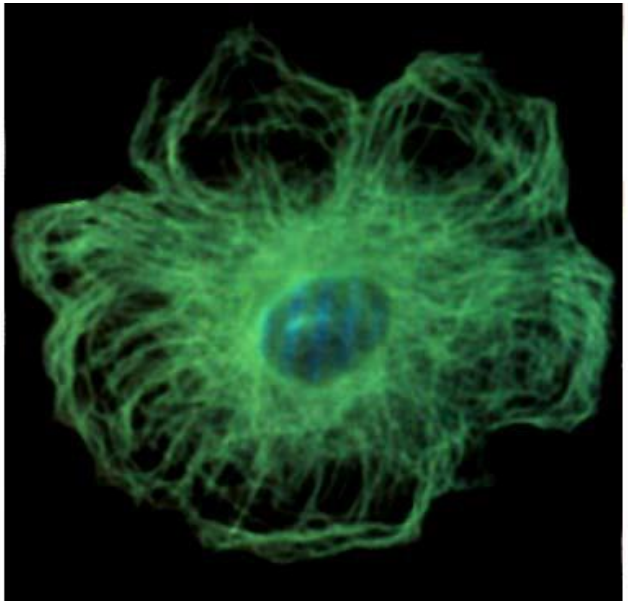
Intermediate filaments

- 10 nm in diameter
- built of a number of different proteins (70), some are tissue-specific
- Provide structural support and mechanical strength

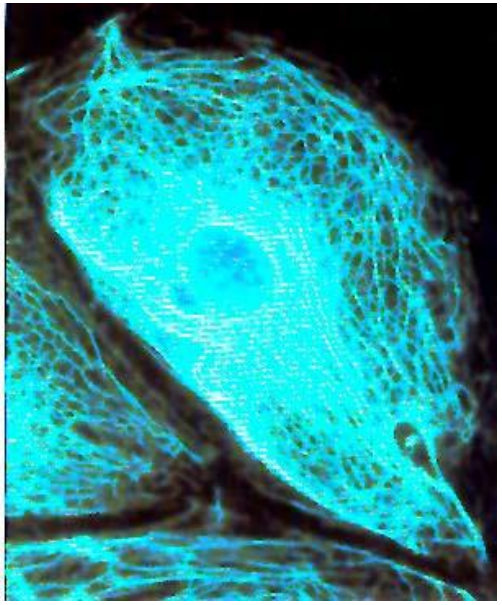
The cytoskeleton



actin filaments



microtubules



intermediate filaments

The different types of filaments can be revealed with different fluorescent compounds:

- Phalloidin – actin
- Fluorescently-labeled subunits (tubulin)
- antibodies